

WHAT IS CLAIMED IS:

1. A method of assembling a double-stranded polynucleotide comprising:

- (a) selecting a partially double-stranded 5 initiating oligonucleotide, wherein said initiating oligonucleotide comprises at least one overhang;
- (b) contacting said partially double-stranded initiating oligonucleotide with a next most terminal double-stranded oligonucleotide, wherein said next most 10 terminal double-stranded oligonucleotide is contiguous with the initiating oligonucleotide and comprises at least one overhang, and wherein at least one overhang of said next most terminal double-stranded oligonucleotide is complementary to at least one overhang of said 15 initiating oligonucleotide;
- (c) repeating (b) to sequentially add the next most terminal double-stranded oligonucleotide to the extended initiating oligonucleotide, whereby said double-stranded polynucleotide is synthesized.

20 2. The method of 1, wherein said initiating oligonucleotide in step (a) has a 3' and a 5' overhang

3. The method of claim 1, wherein said next most terminal oligonucleotide has a 3' and a 5' overhang.

4. A method of assembling a target polynucleotide 25 comprising:

- (a) providing a target polynucleotide sequence;
- (b) identifying at least one partially double-stranded initiating oligonucleotide present in said 30 target polynucleotide, wherein said initiating

oligonucleotide comprises a 5' overhang and a 3' overhang;

- (c) identifying a next most terminal double-stranded oligonucleotide present in said target
5 polynucleotide, wherein said next most terminal double-stranded oligonucleotide is contiguous with the initiating oligonucleotide and comprises a 5' overhang and a 3' overhang, wherein at least one overhang of said next most terminal double-stranded oligonucleotide is
10 complementary to at least one overhang of said initiating oligonucleotide;

(d) contacting said initiating oligonucleotide with said next most terminal double-stranded oligonucleotide under such conditions and for such time
15 suitable for annealing, wherein said initiating sequence is extended; and

(e) repeating (a) through (d) to sequentially add the next most terminal double-stranded oligonucleotide to the extended initiating
20 oligonucleotide, whereby a target polynucleotide is synthesized.

5. The method of claim 4, wherein said initiating oligonucleotide is extended in a bi-directional manner.

25 6. The method of claim 4, wherein said initiating oligonucleotide is extended in a uni-directional manner.

7. The method of claim 4, wherein said initiating oligonucleotide is the 5' most terminal
30 oligonucleotide of said target polypeptide.

8. The method of claim 4, wherein said initiating oligonucleotide is the 3' most terminal oligonucleotide of said target polypeptide.

9. The method of claim 4, wherein the target 5 polynucleotide comprises a sequence that encodes a target polypeptide.

10. The method of claim 9, wherein said target polypeptide is a protein.

11. The method of claim 10, wherein said 10 protein is an enzyme.

12. The method of claim 4, wherein said initiating oligonucleotide comprises a sequence identified by a computer program.

13. The method of claim 12, wherein said 15 computer program comprises an algorithm comprising the following steps:

- (a) parsing a protein sequence codon by codon;
- (b) providing a synthetic gene sequence using E. coli Type II codons;
- 20 (c) parse the sequence of step (b) Q oligomer by Q oligomer into Q length oligonucleotides with X overlap for foward set;
- (d) parse sequence into Q length oligonucleotide form the reverse strand overlapping by X nucleotides;
- 25 (e) determine sequence of REVERSE sequence end "suffer" fragments of Q/2 length; and
- (f) synthesize sequence using array synthesizers.

14. The method of claim 4, wherein said complementary overhang of said next most terminal double-stranded oligonucleotide comprises about fifty percent of the length of the strand having said complementary 5 overhang.

15. The method of claim 14, wherein said strand having said complementary overhang is about 15 to 1000 nucleotides in length.

16. The method of claim 15, wherein said strand having said complementary overhang is about 20 to 500 nucleotides in length.

17. The method of claim 16, wherein said strand having said complementary overhang is about 25 to 100 nucleotides in length.

18. The method of claim 4, wherein the initiating oligonucleotide is attached to a solid support

5 19. A method of assembling a double-stranded polynucleotide, comprising:

(a) chemically synthesizing a first set of oligonucleotides of at least 25 bases comprising a first strand of a double-stranded polynucleotide;

10 (b) chemically synthesizing a second set of oligonucleotides of at least 25 bases comprising a second complementary strand of said double-stranded polynucleotide, each of said oligonucleotides within said second set of said oligonucleotides overlapping with at 15 least one oligonucleotide within said first set of said oligonucleotides; and

(c) annealing said first and second sets of oligonucleotides to produce a double-stranded polynucleotide in the absence of enzymatic synthesis.

20. The method of claim 19, wherein said 5 double-stranded polynucleotide is at least 700 base pairs in length.

21. The method of claim 20, wherein said double-stranded polynucleotide is replication-competent.

22. A method of assembling a double-stranded 10 polynucleotide, comprising:

(a) chemically synthesizing a first set of oligonucleotides comprising a first strand of a double-stranded polynucleotide;

15 (b) chemically synthesizing a second set of oligonucleotides comprising a second complementary strand of said double-stranded polynucleotide, each of said oligonucleotides within said second set of said oligonucleotides overlapping with at least one oligonucleotide within said first set of said 20 oligonucleotides; and

(c) annealing in the presence of MutS protein said first and second sets of oligonucleotides to assemble a double-stranded polynucleotide.

23. A method of assembling a double-stranded 25 polynucleotide comprising:

(a) chemically synthesizing a first set of oligonucleotides comprising a first strand of a double-stranded polynucleotide;

- (b) chemically synthesizing a second set of oligonucleotides comprising a second complementary strand of said double-stranded polynucleotide, each of said oligonucleotides within said second set of said 5 oligonucleotides overlapping with at least one oligonucleotide within said first set of said oligonucleotides, and
- (c) annealing said first and second sets of oligonucleotides to produce a partially double-stranded 10 component polynucleotide;
- (d) repeating steps (a) through (c) to prepare a series of partially double-stranded component polynucleotides;
- (e) selecting at least one partially double-stranded component polynucleotide present in said target 15 polynucleotide to serve as the initiating polynucleotide, wherein said initiating polynucleotide comprises a 5' overhang and a 3' overhang;
- (f) adding the next most terminal component 20 polynucleotide, wherein said next most terminal component polynucleotide comprises at least one overhang that is complementary to at least one overhang of the initiating polynucleotide;
- (g) contacting said initiating polynucleotide 25 with said next most terminal component polynucleotide under such conditions and for such time suitable for annealing; and
- (h) optionally repeating (e) through (g) to sequentially add said next most terminal component 30 polynucleotide to the extended initiating polynucleotide, whereby a target polynucleotide is assembled in the absence of enzymatic polymerization.